FEBS Letters 580 (2006) 6375–6383

Apoptosis-inducing factor (AIF) inhibits protein synthesis by interacting with the eukaryotic translation initiation factor 3 subunit p44 (eIF3g)

Jong-Tae Kim[a,b], Kwang Dong Kim[b], Eun Young Song[b], Hee Gu Lee[b], Jae Wha Kim[b], Jung Woo Kim[c], Suhn-Kee Chae[c], Eunhee Kim[d], Myeong-Sok Lee[a], Young Yang[a], Jong-Seok Lim[a,*]

[a] *Department of Biological Sciences, Research Center for Women's Diseases, Sookmyung Women's University, Chungpa-Dong, Yongsan-Gu, Seoul 140-742, Republic of Korea*
[b] *Laboratory of Cell Biology, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Republic of Korea*
[c] *Division of Life Science, PaiChai University, Daejeon 302-735, Republic of Korea*
[d] *School of Bioscience and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea*

Received 23 August 2006; revised 25 October 2006; accepted 25 October 2006

Available online 3 November 2006

Edited by Richard Marais

Abstract **Apoptosis-inducing factor (AIF) is a ubiquitous FAD-binding flavoprotein comprised of 613 amino acids and plays an important role in caspase-independent apoptosis. During apoptotic induction, AIF is translocated from the mitochondrial intermembrane space to the nucleus, where it interacts with DNA and activates a nuclear endonuclease. By performing a yeast two-hybrid screen with mature AIF, we have isolated the eukaryotic translation initiation factor 3 subunit p44 (eIF3g). Our deletion mutant analysis revealed that the eIF3g N-terminus interacts with the C-terminal region of AIF. The direct interaction between AIF and eIF3g was confirmed in a GST pull-down assay and also verified by the results of co-immunoprecipitation and confocal microscopy studies. Using an in vitro TNT coupled transcription–translation system, we found that mature AIF could inhibit newly-translated protein synthesis and this inhibition was significantly blocked by eIF3g competitively. These results were also confirmed in cells. In addition, mature AIF overexpression specifically resulted in the activation of caspase-7, thereby amplifying the inhibition of protein synthesis including eIF3g cleavage. Our data suggest that eIF3g is one of the cytosolic targets that interacts with mature AIF, and provide insight into the AIF's cellular functions of the inhibition of protein synthesis during apoptosis.**

Keywords: Apoptosis-inducing factor; eIF3; Protein synthesis; Caspase

1. Introduction

Apoptosis plays a critical role in important biological processes such as morphogenesis, tissue homeostasis, and immunity; furthermore, its aberrant activation or impairment may contribute to a number of diseases [1,2]. Cells undergoing apoptosis usually exhibit a characteristic morphology, including apoptotic body formation, fragmentation of the cellular proteins, nuclear and cytoplasmic condensation, and chromosomal DNA cleavage. These characteristics of apoptosis comprise both caspase-dependent and caspase-independent pathways. Caspases are major mediators involved in several death pathways, such as extrinsic death receptor-mediated or intrinsic mitochondria-mediated signaling. Caspase activation affects a number of substrates that have important biological functions, leading to the loss of their functional roles.

Apoptosis-inducing factor (AIF) has been identified as an apoptogenic mitochondrial intermembrane protein and has been demonstrated to play roles as a caspase-independent apoptotic factor [3–7]. Similar to cytochrome *c* (Cyt-*c*), AIF is likely to be a phylogenetically ancient, bifunctional protein that exhibits an oxidoreductase function [8–10]. The AIF precursor protein (613 amino acids) contains an N-terminal mitochondrial localization sequence (MLS) by which the protein is guided to the mitochondrial intermembrane space (MIS). It is synthesized in the cytoplasmic ribosomes and then imported into the MIS, where the MLS is removed by the calcium-activated protease calpain [5,11]. The large C-terminal domain of AIF shares significant homology with oxidoreductases from vertebrates to invertebrates, and it has mitochondrial NADH oxidase activity [9]. During apoptotic induction, such as following treatment with staurosporin or cisplatin, the mitochondria release mature AIF and other soluble proteins of the intermembrane space such as Cyt-*c*. When cells are transfected with an AIF deletion mutant lacking a MLS, AIF accumulates in the extramitochondrial compartments, in particular the nuclei. This results in chromatin condensation and cell death [12]. In addition, some evidence suggests that sequence-independent DNA binding of AIF is required for its apoptogenic functions [13,14]. In cells treated with apoptotic stimuli, endogenous AIF becomes co-localized with DNA at an early stage of nuclear morphological change. Structure-based mutagenesis reveals that DNA-binding defective mutants of AIF fail to induce cell death, while retaining nuclear translocation characteristics. One major difference between AIF-induced and Cyt-*c*/caspase/

*Corresponding author. Fax: +82 2 2077 7322.
E-mail address: jslim@sookmyung.ac.kr (J.-S. Lim).

Abbreviations: AIF, apoptosis-inducing factor; eIF, eukaryotic translation initiation factor; MLS, mitochondrial localization sequence; RRM, RNA recognition motif

0014-5793/$32.00
doi:10.1016/j.febslet.2006.10.049

CAD-induced nuclear apoptosis involves the morphology of chromatin condensation. AIF causes only a partial condensation without shrinkage, whereas CAD (caspase-activated DNase) induces a more advanced condensation pattern [7]. Another difference involves the degree of chromatin degradation induced by AIF and CAD. AIF induces (caspase-independently) large-scale (50 kbp) DNA fragmentation [12,15], whereas CAD triggers a more classical oligonucleosomal pattern of DNA fragmentation, leading to the production of multiples of 200 bp. However, the exact mechanism by which AIF induces chromatin condensation and DNA fragmentation has yet to be elucidated. To date, AIF has been reported to interact with heat shock protein 70 (HSP70), an anti-apoptotic protein that binds to Apaf-1, forming the apoptosome [16–18], and cyclophilin A (CypA) which binds with immunosuppressive cyclosporin A (CyA) [19]. It has been shown that HSP70 inhibits the translocation of AIF to the nucleus and AIF-induced chromatin condensation of purified nuclei, and that apoptosis is inefficiently carried out in CypA-knockout cells or with AIF mutants lacking the CypA-binding domain.

In order to explore possible interactions related to the molecular function of AIF in the mitochondria, cytosol, and nucleus, we performed a yeast two-hybrid screen using the mutant AIFΔ1–101 (a mature AIF that translocates to the nucleus) as the bait. One of the positive clones, which encoded a subunit of eukaryotic translation initiation factor 3, eIF3g, was analyzed in more depth. eIF3, which comprises the eIF machinery, is a large translation initiation complex containing at least 13 subunits [20,21]. eIF3 plays a central role in the binding of the initiator methionyl-tRNA and mRNA with the 40S ribosomal subunit to initiate translation [22]. In this study, we demonstrate that mature AIF released from mitochondria interacts specifically with eIF3g in the eIF3 complex, thereby blocking translation initiation. These data make it clear that eIF3g, though its exact role in apoptosis is yet undefined, is one of the cytosolic factors that bind to the mature form of AIF.

2. Materials and methods

2.1. Cell culture and treatment

Jurkat or MCF-7 cells were cultured in RPMI 1640 (Sigma, St. Louis, MO), and HeLa and HEK293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco-BRL), 1% penicillin, streptomycin, and 2 mM glutamine in a humidified 5% CO_2 incubator.

2.2. Antibodies

Antibodies used in this study are as follows: rabbit anti-AIF and anti-GFP antibody (Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-GST antibody (Molecular Probes, Eugene, OR), mouse anti-HA and anti-α-tubulin antibody, TRITC- or HRP-conjugated secondary antibody (Sigma), mouse anti-caspase-3 antibody (Calbiochem, San Diego, CA), rabbit anti-caspase-6 and anti-caspase-7 antibody (Cell Signaling Technology, Beverly, MA). Rabbit anti-eIF3g antibody was kindly provided by Dr. Tang K. Tang (Institute of Biomedical Sciences, Taiwan), and we also generated polyclonal anti-eIF3g antibody from mouse via the injection of purified recombinant eIF3g protein.

2.3. Plasmid construction

In order to analyze the interaction between AIF and eIF3g, using a yeast two-hybrid system, the DNA fragments encoding AIF wt (amino acids 1–613), -mat (Δ1–101), -N (1–153), -M1 (102–323), -M2 (102–439), -M3 (310–439), -C2 (373–613), -C1 (440–613), and -C3 (500–613) were cloned into the pLexA vector (Clontech, Palo Alto, CA). eIF3g wt (amino acids 1–320), -Δ*R* (Δ230–320), -C1 (230–320), -C2 (114–320), and -Δ*N* (Δ1–74) were cloned into the pB42AD vector. For in vitro coupled transcription–translation analysis, the cDNAs of AIF, eIF3g, eIF3i, eIF3j, GFP, and luciferase were cloned into the pcDNA3 (or pcDNA3HA) vector (Invitrogen, Carlsbad, CA). To generate recombinant glutathione-*S*-transferase (GST) fusion proteins, DNA fragments were cloned into the pGEX-5X-1 vector (Amersham Pharmacia Biotech, Piscataway, NJ). For cellular expression and interaction analyses in mammalian cells, eIF3g was cloned into the pEBG vector (New England BioLabs, Beverly, MA) and the pEGFPC2 vector (Clontech). All cDNA constructs were verified by automatic DNA sequencing and their protein expression was confirmed by Western blotting.

2.4. Yeast two-hybrid screening and β-galactosidase assay

The LexA-based yeast two-hybrid system was used to screen for AIF-interacting proteins. The yeast used in this study was *Saccharomyces cerevisiae* strain EGY48 (*MATα, his3, trp1, ura3, LEU2::LexA*$_{op(x6)}$/pSH18-34). Cloned pLexA-AIFmat plasmid was transformed into the yeast via lithium acetate-mediated transformation, and the clones growing in selection media were cultured. After examination of Lex-AIFmat's expression and self-transcription activity, the HeLa cDNA library DNA (10 μg) was transformed into the LexA-AIFmat-yeasts, and positive clones were isolated using a colony-lift filter assay according to the Clontech Matchmaker manual (Clontech). Library plasmids from positive clones were extracted and the plasmids were identified by DNA sequencing and the NCBI Blast Program. To confirm the interaction of AIF and eIF3g, yeasts were co-transformed with the different AIF and eIF3g wt or deletion mutants and a β-galactosidase assay was performed. In brief, yeasts containing different sets of plasmids were cultured until mid-log phase was achieved. Yeasts were lysed with liquid nitrogen and the fluorometric reaction was initiated by adding the substrate ONPG in Z buffer (60 mM $Na_2HPO_4 \cdot 12H_2O$, 40 mM $NaH_2PO_4 \cdot 2H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, pH 7.0) containing 2-mercaptoethanol. β-Galactosidase activity was calculated according to the Clontech Matchmaker manual.

2.5. GST pull-down assay

GST, GST-AIF, or GST-eIF3g plasmids were transformed into *E. coli* BL21 (DE3) and the protein expression was induced by the addition of IPTG up to a concentration of 1 mM at 37 °C for 4 h. The GST or GST-fusion proteins were extracted using a lysis buffer (20 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM PMSF). The lysates were immobilized onto glutathione-Sepharose 4B resin (Amersham) at 4 °C for 4 h. In order to generate ^{35}S-Met-labeled proteins using the TNT coupled transcription-translation system (Promega, Madison, WI), pcDNA3 constructs and ^{35}S-methionine (Perkin-Elmer Life Sciences, Boston, MA) were added to the TNT reticulocyte lysates, and the lysates were incubated at 30 °C for 90 min. For the GST pull-down assay, the immobilized GST or GST-fusion proteins were incubated with mammalian cell lysates (1 mg) or in vitro ^{35}S-labeled proteins at 4 °C for 4 h. After pull-down, proteins remaining on the Sepharose beads were resuspended with SDS sample buffer, resolved using SDS–PAGE, and analyzed by Western blotting or autoradiography.

2.6. In vitro translation assay

GST or GST-fusion proteins immobilized onto Sepharose resin were eluted with glutathione elution buffer (50 mM Tris–HCl, pH 9.0, 20 mM glutathione) according to manufacturer's instructions. The eluted GST or GST-fusion proteins were then incubated with TNT reticulocyte lysate at 4 °C for 4 h. Both T7 luciferase DNA and 10 μCi of ^{35}S-Met were added to the recombinant protein–reticulocyte mixtures, and then reincubated for at 30 °C for 90 min. Alternatively, ^{35}S-Met, pcDNA3-AIFmat, and/or pcDNA3-eIF3g were added to the TNT reticulocyte lysate and incubated at 30 °C for 1 h. Then, pcDNA3-GFP plasmid was added and the mixtures were reincubated for 1 h. After the reaction was stopped by SDS sample buffer, the newly synthesized ^{35}S-Met-labeled proteins were analyzed by SDS–PAGE and autoradiography.

2.7. Transfection, immunoprecipitation, and Western blotting

Cells were distributed into 6-well or 100 mm plates and transfected with various plasmids using Lipofectamine transfection reagent (Invitrogen). After 24–48 h, the cells were washed with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4, pH 7.4), lysed by RIPA lysis buffer (1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 10 mM Tris–HCl, pH 7.6, 1 mM EDTA, 50 mM sodium fluoride, 1 mM PMSF, and protease cocktail), and then incubated on ice for 30 min. After the removal of cell debris via centrifugation at 12000 rpm for 10 min, protein content was determined using the Bio-Rad protein assay system (Bio-Rad Laboratories, Hercules, CA). To degrade nucleic acids in cell lysates, lysates were treated together with DNase and RNase A at 37 °C for 1 h. For immunoprecipitation, anti-AIF or anti-GST antibodies (2 μg) were added into the cell lysates (1 mg) at 4 °C for 4 h, and then protein-A/G agarose was added and the lysates were reincubated for 2 h. After centrifugation, precipitated immune complexes were resuspended in SDS sample buffer. The quantified lysates or immune complexes were subjected to SDS–PAGE, and subsequently transferred onto Hybond PVDF membranes (Amersham). The membranes were blocked with 5% non-fat dry milk in PBS, and then incubated with the appropriate primary and HRP-conjugated secondary antibodies at room temperature for 2 h. Following another extensive washing, protein bands were visualized using an enhanced chemiluminescence (ECL) Western blotting detection kit (Amersham).

2.8. Reverse-transcriptase (RT)-PCR analysis

Total RNA was extracted from the transfected cells by the acid guanidinium thiocyanate-based extraction method and the RNAs were dissolved in 0.1% diethylpyrocarbonate-treated water. cDNA was synthesized from RNA using a ProSTAR RT-PCR kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. PCR-amplified cDNAs were separated on a 1.2% agarose gel and stained with ethidium bromide.

2.9. Detection of caspase activation

To detect active caspase in apoptotic cells, we used a Carboxyfluorescein FLICA Apoptosis Detection Kit (Immunochemistry Technologies, Bloomington, MN) according to manufacturer's instructions. In brief, MCF-7 cells growing on coverslips were transfected with pcDNA3-AIFmat and then treated with FAM-DEVD-fmk, an active caspase-3/7 substrate, for 1 h in a CO_2 incubator. After washing with DMEM, the cells were stained with Hoechst dye and anti-AIF antibody. To measure caspase activity directly, MCF-7 cells were transfected with pcDNA3 constructs and treated with Z-DEVD-fmk (0.5 μM, Calbiochem) or cisplatin (10 μg, Sigma) for 1 day. Cells were harvested, lysed, and incubated with Caspase-Glo 3/7 Reagent (Promega) for 10 min at room temperature, and the luminescence was then assessed using a plate-reading luminometer (Turner Designs, Sunnyvale, CA).

2.10. Immunofluorescence confocal microscopy

Cells grown on coverslips were transfected with pEGFP or pEGFP-eIF3g, and the cells were treated with cisplatin for 1 day. The cells were washed with PBS, fixed in 4% paraformaldehyde for 20 min, and permeabilized with 0.3% Triton X-100/PBS for 10 min. After washing, cells were blocked in 1% BSA/PBS for 30 min at room temperature, washed, and incubated with anti-AIF antibody for 1 h, followed by incubation with TRITC-conjugated anti-goat antibody. Cells were then treated with DAPI (Calbiochem), and washed with PBS containing 0.1% Triton X-100. Cell-containing coverslips were mounted onto glass slides with Vectashield mounting medium (Vector Laboratories, Burlingame, CA), and visualized using a Zeiss confocal microscope LSM510META (Carl Zeiss, Jena, Germany) at 40× magnification. The confocal images were captured by the Zeiss LSM Image Browser program.

3. Results

3.1. AIFmat interacts with eIF3g in yeast two-hybrid system

In order to gain an understanding of the mechanism underlying the function of AIF, we initially searched for AIF binding proteins in a human HeLa cDNA library, using the yeast two-hybrid screening method. AIFmat cDNA, the mature form of AIF which translocates from mitochondria to the nucleus during apoptosis, was cloned into the pLexA vector, and the expression of LexA-AIFmat protein was examined by Western blotting. Before library screening, we also examined the self-transcription activity of AIFmat and found that AIFmat expression alone did not activate the reporter gene, β-galactosidase. According to the results of DNA sequencing and NCBI database searches from the AIFmat-interacting positive clones, the nucleotide sequences of 4 of the 12 cDNA clones isolated actually encoded for the eukaryotic translation initiation factor 3 subunit p44 (eIF3g) (data not shown).

3.2. AIFmat interacts with eIF3g in vitro

To determine whether or not eIF3g interacts directly with mature AIF, we performed an in vitro binding study using recombinant GST-fusion proteins and in vitro ^{35}S-Met-labeled proteins. Recombinant GST-AIFmat and GST-eIF3g fusion proteins were generated in *E. coli*, and their protein expressions were verified via Western blot analysis. In order to analyze protein interactions occurring between AIFmat and eIF3g, immobilized GST fusion proteins were incubated with ^{35}S-Met-labeled proteins. After the GST pull-down, pelleted proteins were subjected to SDS–PAGE, followed by autoradiography (Fig. 1A and B). AIF and AIFmat proteins interacted strongly with eIF3g, but not with eIF3i, eIF3j, or GST alone. The eIF3i and eIF3j subunits have been demonstrated to form the eIF3 complex with eIF3g. The *S. cerevisiae* eIF3g (TIF35) has been previously demonstrated to interact specifically with eIF3b (PRT1) and eIF3i (TIF34) in the yeast two-hybrid system [13–15]. Alternatively, when mammalian cell lysates from HeLa, Jurkat, or HEK293 cells were incubated with GST-eIF3g protein, AIF protein was detected by Western blot using anti-AIF antibody, indicating that this endogenous AIF protein interacted strongly with the eIF3g protein (Fig. 1C). Along with the results from the yeast two-hybrid assay, these results bolster the assertion that eIF3g interacts specifically with AIF in vitro.

3.3. AIFmat interacts with eIF3g near the nucleus

The interaction of AIF and eIF3g was confirmed by immunoprecipitation assay in HEK293 cells. To test whether cellular AIF associates with eIF3g in vivo, cells were transfected with AIFmat and eIF3g fused with GST. When the lysates from these transfectants were examined, endogenous AIF, exogenous AIFmat, and GST-eIF3g protein expression was detected by Western blotting. Cell lysates were initially immunoprecipitated with anti-GST antibody and co-precipitated proteins were then detected using anti-AIF antibody (Fig. 2A). AIF and AIFmat were detected in GST-eIF3g lysate, but not in GST lysate. Also, when the lysates were immunoprecipitated with anti-AIF antibody, co-precipitated proteins were detected as fusion proteins by Western blot, using anti-GST antibody. Alternatively, in order to obtain direct evidence for in vivo interaction, immunoprecipitation using anti-AIF antibody was performed in the AIFmat-transfected lysates. In this experiment, because eIF3g contains an RNA recognition motif (RRM) and AIF binds to DNA, cell lysates were treated with nucleases to exclude the nucleic acids (Fig. 2B). The direct interaction of AIF with eIF3g was significantly detected even

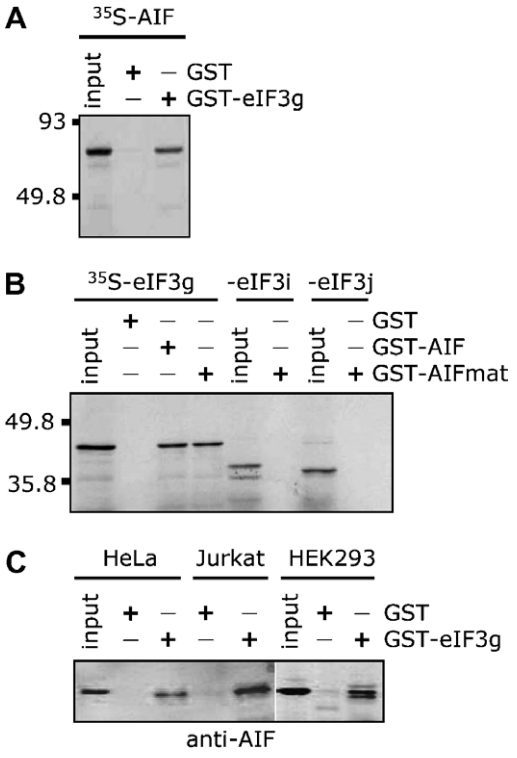

Fig. 1. AIF interacts with eIF3g in vitro. Purified recombinant GST or GST-fusion proteins that were generated from *E. coli* were immobilized onto glutathione resin, and ^{35}S-Met-labeled proteins were generated using a TNT coupled transcription–translation reticulocyte lysate system. These resin-bound GST or GST-fusion proteins were incubated with ^{35}S-proteins or mammalian cell lysates, and a GST pull-down assay was performed. (A) GST or GST-eIF3g protein was incubated with ^{35}S-labeled AIF. After GST pull-down, protein pellets were resuspended in SDS sample buffer and subjected to SDS–PAGE followed by autoradiography. (B) GST-AIF or GST-AIFmat protein was incubated with ^{35}S-eIF3g, -eIF3i, or -eIF3j protein, and GST pull-down was performed. ^{35}S-eIF3g protein was co-precipitated with GST-AIF and GST-AIFmat, but ^{35}S-eIF3i and -eIF3j were not co-precipitated with GST-AIFmat. (C) GST-eIF3g protein was incubated with mammalian cell lysates, instead of ^{35}S-labeled proteins. After the GST pull-down, the pellets were subjected to SDS–PAGE followed by Western blotting for AIF. Input was 10% of incubation lysates.

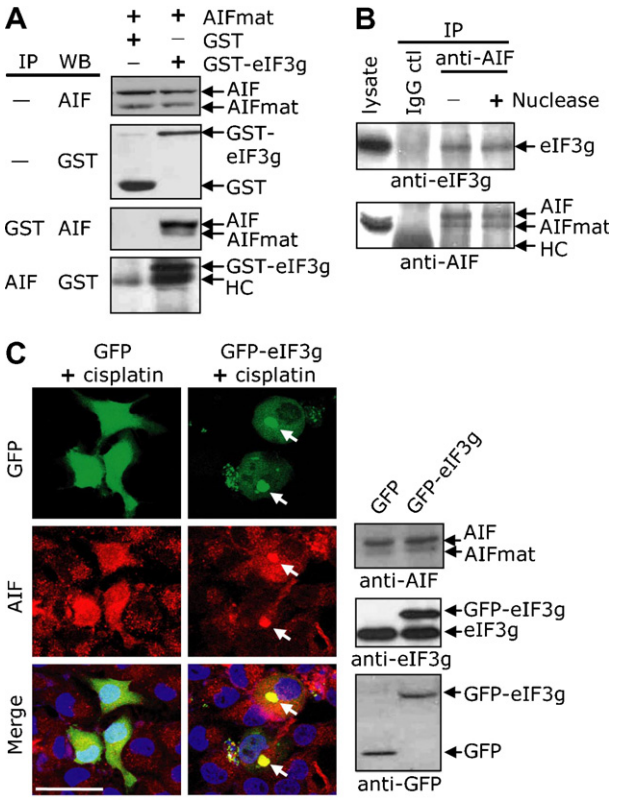

Fig. 2. AIF directly interacts with eIF3g near nucleus. (A) HEK293 cells were transiently transfected with pcDNA3-AIFmat and pEBG (GST)-eIF3g, and the cell lysates were prepared and immunoprecipitated with anti-GST or anti-AIF antibody. After immunoprecipitation, the immune complexes were resuspended in SDS sample buffer, subjected to SDS–PAGE, and analyzed by Western blotting. AIF and AIFmat were pulled down with GST-eIF3g by using an anti-GST antibody, and GST-eIF3g was pulled down with AIFs by using an anti-AIF antibody. (B) AIFmat-transfected cell lysates were incubated with or without nucleases to degrade their nucleic acids, and these lysates were immunoprecipitated with anti-AIF antibody. eIF3g was detected in the AIF-immunoprecipitated pellets regardless of pretreatment with nuclease, but not detected in the pellet immunoprecipitated with IgG control antibody. HC, heavy chain of immunoglobulin. (C) Cells were transfected with GFP or GFP-eIF3g and were treated with cisplatin for 1 day. For immunofluorescence analysis, the cells were fixed and stained with anti-AIF antibody (red) and DAPI (nuclei, blue) as described in Section 2. After examination of protein expression using Western blotting, cellular localization of AIF and eIF3g were analyzed using a confocal microscope. Arrows indicate the co-localization of AIF and eIF3g. Bar, 50 μm.

in the presence of nucleases. This cellular interaction of AIFmat and eIF3g was viewed on a confocal microscope. To do this, HEK293 cells were transfected with GFP or GFP-eIF3g plasmid and the cells were treated with cisplatin to induce apoptosis. As shown in Fig. 2C, GFP proteins appeared to be equally distributed within the cells, but GFP-eIF3g proteins were found to be mainly distributed throughout the cytosol, while being only weakly distributed in the nucleus. In the case of AIF, nuclear translocation was partially observed with the induction of apoptosis using 5 μg/ml cisplatin. Importantly, under the same conditions, the region of mature AIF and eIF3g co-localization was identified near the nuclear membrane, presumably in the endoplasmic reticulum (Fig. 2C, arrows). Consistent with these findings, the co-localization of GFP-AIF155–613 with endogenous eIF3g was also detected by anti-eIF3g antibody (data not shown). This co-localization indicates that mature AIF might associate with eIF3g near the nucleus in apoptotic induction. Therefore, our results indicate that mature AIF proteins do indeed interact with eIF3g in vivo.

3.4. The AIF C-terminus and eIF3g N-terminus are important for their protein binding

In order to construct a map of the region of AIF which binds to eIF3g, we generated various protein fragments of AIF or eIF3g, forming LexA-fusion or B42-fusion proteins (Fig. 3A). These deletion mutant constructs of AIF and eIF3g were transformed into yeast EGY48, and their protein interaction was determined by measurement of the expression levels of the lacZ reporter gene. As shown in Fig. 3B, AIF and AIFmat exhibited strong binding to eIF3g, whereas AIF-N, -M1, -M2, and -M3 (deletion mutants of the C-terminal residues) revealed no interaction with eIF3g. However, the C-terminal fragments of AIF that contained the nuclear localization sequence 2 (NLS2), encompassing amino acids 373–613 (AIF-C2) and 440-613 (AIF-C1), both interacted significantly with eIF3g. On the

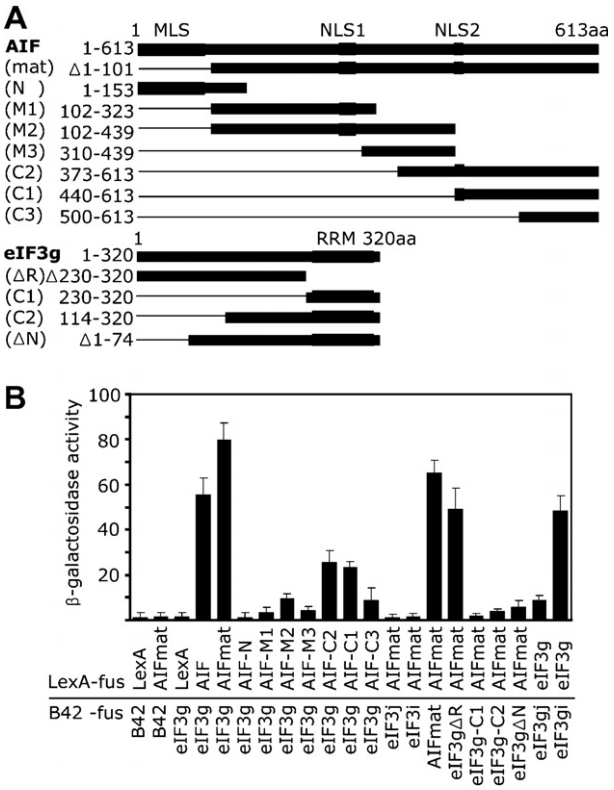

Fig. 3. Determination of binding site between AIF and eIF3g. (A) Schematic diagrams of AIF and eIF3g deletion mutants. AIF contains a MLS at the N-terminus and two NLS, and eIF3g contains a RRM at C-terminus. (B) β-Galactosidase reporter assay for the protein interaction between AIF and eIF3g. Various combinations of pLexA- and pB42-constructs plasmids were co-transformed into EGY48 yeast, and colonies growing in selection media were cultured. Exponentially-growing yeast cells were lysed and β-galactosidase activity was determined as described in Section 2. β-Galactosidase activity indicates that the LexA-fusion protein interacts with the B42-fusion protein.

other hand, AIF displayed no interaction with other eIF3 subunits such as eIF3i and eIF3j (This was verified in Fig. 1B), whereas AIFmat interacted strongly with another AIFmat protein. When we expressed the deletion mutants of eIF3g, AIFmat interacted only with eIF3gΔR (Δ230–320), which does not harbor the RRM, and not with eIF3g-C1 (230–320), eIF3g-C2 (114–320), eIF3gΔ*N* (Δ1–74), or the pB42AD vector alone. The interaction of eIF3g with the eIF3i subunit, but not the eIF3j subunit, was confirmed for positive interactions, as reported by others [15–17]. These results indicate that the region directly interacting with the C-terminal region of AIF is the N-terminal region of eIF3g.

3.5. AIF inhibits de novo protein synthesis by binding to eIF3g

As the eIF3 complex is important for de novo protein synthesis, and eIF3g is required for eIF3's function, we therefore examined whether the interaction of AIFmat with eIF3g plays a role in protein biosynthesis. When GST-AIFmat protein was incubated with TNT reticulocyte lysate, the synthesis of ^{35}S-Met-labeled luciferase protein by luciferase DNA was inhibited in a dose-dependent manner, but GST protein did not affect the synthesis (Fig. 4A). In the TNT reticulocyte lysates, AIF protein was not detected by Western blotting (data not shown), excluding the possible contribution of AIF from TNT reticulocyte lysate. Furthermore, upon the addition of increasing amounts of GST-eIF3g protein to the reaction mixtures containing GST-AIFmat, the repressed luciferase synthesis was significantly recovered (Fig. 4B). The recovery of translation was almost complete after the addition of GST-eIF3g in an equal proportion to GST-AIFmat protein, and this specificity was also verified by GST as a negative control. Alternatively, when AIFmat DNA was used instead of recombinant protein, ^{35}S-Met-GFP synthesis by GFP DNA added later was blocked by the synthesis of AIFmat by the previously-add DNA, resulting in a time-dependent decrease in GFP protein expression (Fig. 4C). Also, as shown in Fig. 4D, when AIFmat and eIF3g DNAs were co-expressed in TNT reticulocyte lysate (lane 1), ^{35}S-Met-GFP synthesis by GFP DNA was similar to that in the presence of eIF3g alone (lane 2) or in mock control (lane 4), whereas it was significantly inhibited in AIFmat-expressing reticulocyte lysate (lane 3). Taken together, these results strongly suggest that mature AIF associates with its interacting protein, eIF3g, thereby blocking de novo protein synthesis.

To confirm AIFmat's function in cellular protein synthesis, HEK293 cells were transfected with AIF mutants and GFP plasmids, and the expression level of GFP protein was analyzed by Western blotting (Fig. 4E and F). When AIFmat or AIF-C1, both of which are able to bind eIF3g, was overexpressed, GFP protein expression decreased in the cell lysates in an AIF dose-dependent manner. However, the inhibition of protein synthesis was not found when AIF-M2, which does not bind to eIF3g, was overexpressed. It is noteworthy that the attenuation of GFP expression occurred at the level of protein synthesis, not at the mRNA level. Similar to the in vitro results with eIF3g, eIF3g expression in HEK293 cells recovered GFP expression repressed by AIFmat expression (Fig. 4F). However, AIF, an immature form of AIF that is localized to the mitochondrial intermembrane space, did not affect GFP expression level (data not shown). These results indicate that mature AIF from mitochondria specifically interacts with eIF3g subunit of eIF3 complex, thereby blocking protein synthesis.

3.6. AIFmat is involved in caspase-7 activation

As it has been established that caspase activation modifies substrates such as eIF2-α, eIF3j, and eIF4B, constituents of the eIF complex, and plays an important role in protein synthesis, we attempted to determine whether the AIFmat-mediated inhibition of translation is related to caspase activity. For this study, cells were transfected with GFP and AIFmat, followed by the addition of Z-DEVD-fmk, a caspase-3/7 inhibitor. Finally, cells were treated with cisplatin as an apoptosis-inducing agent. As shown in Fig. 5A, cisplatin treatment induced relatively weak caspase activation, resulting in the inhibition of GFP expression (lanes 2 and 3). Interestingly, the cisplatin-induced translation inhibition and caspase-3 activation were amplified by the exogenous expression of AIFmat (lanes 5 and 6). Decreased GFP expression, by either cisplatin alone or cisplatin in combination with exogenous AIFmat, was recovered via treatment with Z-DEVD-fmk (lanes 4 and 7). More importantly, the inhibition of translation by exogenous AIFmat alone was also significantly recovered by treatment with Z-DEVD-fmk (lane 9), suggesting the involvement of caspase activation in the AIFmat-mediated inhibition of

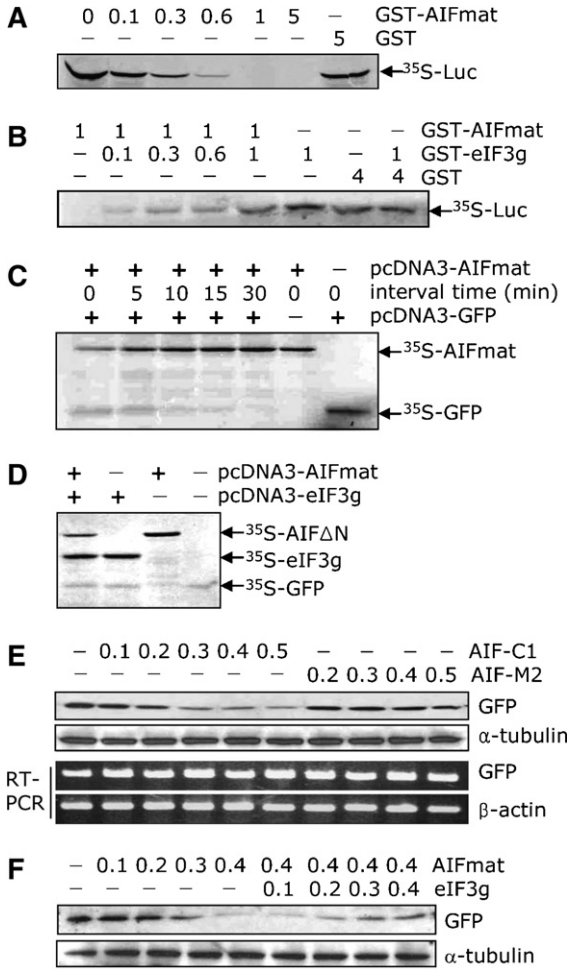

Fig. 4. AIFmat inhibits protein synthesis by binding to eIF3g. (A) After purified recombinant GST or GST-AIFmat was incubated with TNT coupled transcription–translation reticulocyte lysate for protein interaction, ^{35}S-Met and pcDNA3-luciferase DNA were added and reincubated for protein synthesis. The reaction mixtures were then analyzed by SDS–PAGE followed by autoradiography for ^{35}S-luciferase synthesis. GST-AIFmat addition dose-dependently inhibited the Luc synthesis, but GST alone did not. (B) GST, GST-AIFmat, and/or GST-eIF3g were incubated with the TNT reticulocyte lysate for their protein interaction, then this mixtures were reincubated with luciferase DNA and ^{35}S-Met, and the ^{35}S-Luc synthesis was analyzed by autoradiography. GST-eIF3g rescued the Luc synthesis repressed by GST-AIFmat. (C) Instead of recombinant protein addition into the TNT lysate, pcDNA3-constructs were added to confirm the translation inhibition. pcDNA3-AIFmat and ^{35}S-Met were added and incubated at 30 °C for the described time interval, and then GFP DNA was added and reincubated for 1 h. Presynthesized AIFmat protein time-dependently blocked the protein synthesis by GFP DNA added later. (D) AIFmat and eIF3g DNAs were added together or alone with ^{35}S-Met into the TNT reticulocyte lysates, which were incubated to generate protein for 1 h. Following this, GFP DNA was added and the lysates reincubated for 1 h. As above, AIFmat blocked GFP protein synthesis, but the addition of both AIFmat and eIF3g did not. (E) HEK293 cells were transfected with GFP and AIF mutants, and analyzed for their GFP expression by Western blotting and RT-PCR. GFP expression was dose-dependently inhibited by AIF-C1, but not by AIF-M2. Also, to compare the expression levels of RNA and protein, RNA was extracted and RT-PCR was performed. Differential expression of GFP protein depends on the AIF-C1 expression, while RT-PCR for GFP mRNA shows that mRNA is expressed at the same densities. α-tubulin or β-actin was used as a loading control. (F) After transfection with GFP, AIFmat, and eIF3g, cell lysates were analyzed by Western blotting for GFP expression. The decreased GFP expression by AIFmat was dose-dependently recovered by eIF3g.

translation. Interestingly, significant cleavage of eIF3g was observed after treatment with cisplatin and AIFmat, and this cleavage was inhibited in the presence of Z-DEVD-fmk (lanes 5–7). We observed that this cleavage of eIF3g upon apoptosis induction occurred in several cell lines (data not shown). Additionally, when we determined the caspase activation in HEK293 cells transfected with AIFmat or AIF mutants, the reduction of procaspase-7, an indicator of caspase-7 activation, was clearly observed in cells transfected with AIFmat and, to a lesser extent, in AIF-C1-cells (Fig. 5B). However, this was not observed in cells transfected with AIF-M2, which shows no binding activity to eIF3g. Caspase-3 activation, however, was not detected in any of the transfected cells. Using MCF-7 cells, which are deficient in caspase-3 expression, we examined whether casapse-7 can be activated in AIFmat-overexpressing cells. Cells were transfected with AIFmat and labeled with FAM-DEVD-fmk, an active caspase-3/7 substrate. As shown in Fig. 5C, caspase-7 activity was detected in AIFmat-cells, but not in mock-transfected cells. Moreover, direct detection of caspase activities using a proluminescent substrate revealed that caspase-7 activity was induced in MCF-7 cells transfected with AIFmat at more than 25% of control level, and that it could be inhibited by treatment with a caspase-3/7 inhibitor, Z-DEVD-fmk (Fig. 5D). Finally, the increase of cisplatin-mediated cell death was observed after AIFmat transfection, suggesting the involvement of caspase activation by AIFmat expression. At the same time, co-localization of AIF and eIF3g in nucleus of apoptotic cell was also confirmed (data not shown).

Collectively, these data suggest that mature AIF released from mitochondria during apoptosis interacts with eIF3g, and thereby inhibits the eIF3 machinery and protein synthesis, and activates casapse-7 to amplify apoptosis.

4. Discussion

In our current study, we attempted to identify proteins that interact with the mature AIF and isolated cDNA clones which encode eIF3g protein. eIF3 is the largest of the mammalian translation initiation factors, approximately 600 kDa, and appears to play a central role in the initiation of translation. It was originally isolated from rabbit reticulocyte lysates, and was found to contain at least 13 different protein subunits [20,22–24]. eIF3 has been shown to bind to 40S ribosomal subunits, stabilize initiator methionyl-tRNA binding to the 40S subunits, and participate in mRNA binding via interaction with eIF4G [25]. eIF3g, the p44 subunit of the eIF3 complex, has been reported to strongly associate with eIF3a (p170) and bind to rRNA through a RRM domain at the C-terminal region [21]. In *S. cerevisiae* yeast, it has been demonstrated that eIF3g interacts with eIF4B, and that the central part of eIF3g spanning amino-acids 66–173 is involved in binding to eIF4B or eIF3i (p36), whereas the C-terminal part of eIF3g contributes to neither eIF4B nor eIF3i binding [26]. Moreover, it was reported that eIF3g interacts directly with erythroid protein 4.1 (4.1R), and that the RRM of eIF3g is involved in this binding [27]. Interestingly, the depletion of eIF3g from rabbit reticulocyte lysate by the addition of anti-eIF3g antibody was found to result in a reduction of the lysate's ability to synthesize proteins efficiently. In our study, we have shown that, un-

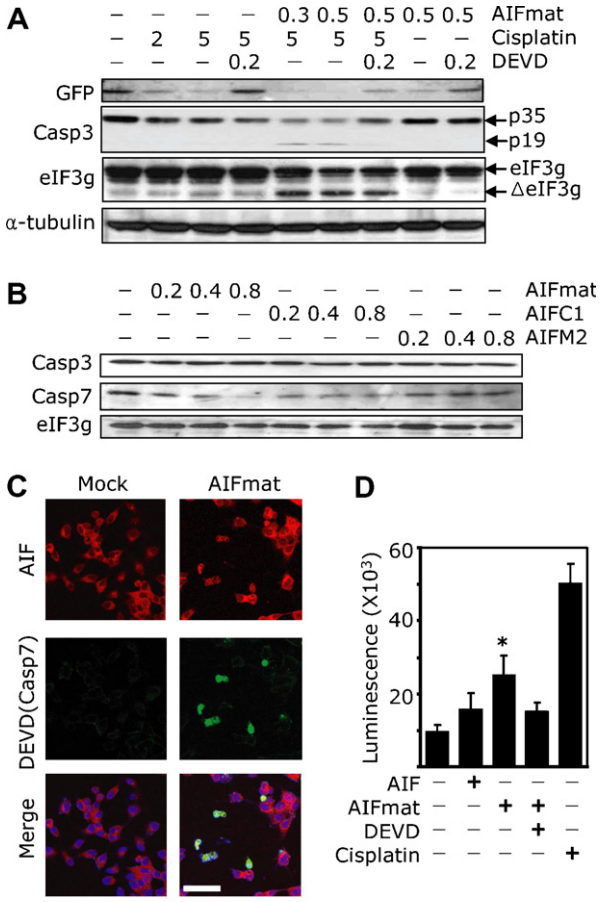

Fig. 5. AIFmat activates caspase-7, not caspase-3. (A) After HEK293 cells were transfected with GFP and AIFmat for 1 day, cells were treated with cisplatin and/or Z-DEVD-fmk (caspase-3/7 inhibitor) as indicated. Cisplatin and AIFmat inhibited the GFP expression and this expression was recovered by caspase inhibitor. AIFmat more strongly blocked the GFP protein synthesis with cisplatin treatment (lanes 5 and 6), and the decreased GFP expression was significantly recovered by Z-DEVD-fmk (lane 7). Under this condition, eIF3g was significantly cleaved concomitantly with caspase activation. Also, when AIFmat was expressed without cisplatin treatment, the decreased GFP expression was recovered by Z-DEVD-fmk, indicating that AIFmat is related to caspase activity. (B) Cells were transfected with AIF mutants and analyzed by Western blotting for caspases. Caspase-7 was activated by AIFmat and AIF-C1, whereas caspase-3 was not activated by AIF mutants. (C) MCF-7 cells on coverslips were transfected with pcDNA3 or pcDNA3-AIFmat, and the cells were incubated with FAM-DEVD-fmk (caspase-3/7 substrate). Then, the cells were fixed and stained with Hoechst dye (blue) and anti-AIF antibody (red). AIF, caspase-7, and nuclear stain were detected using confocal microscopy. Bright green spots in AIFmat panels indicate that casapse-7 is activated. bar, 50 μm. (D) MCF-7 cells were transfected with AIF or AIFmat and treated with Z-DEVD-fmk. Cells were harvested, lysed and incubated with Caspase-Glo 3/7 Reagent (a caspase-3/7 substrate). The luminescence of each sample in duplicate was measured with a plate-reading luminometer. Results are means ± S.E.M. of three independent experiments. *, $P < 0.05$, AIF versus AIFΔN.

like its interaction with 4.1R, eIF3g does not require the presence of the RRM for association with AIF. We could clearly demonstrate that the AIF C-terminus interacts with the eIF3g N-terminus, which lacks the RRM motif, to inhibit protein synthesis in vivo and in vitro. Furthermore, the addition of increasing amounts of mature AIF to the lysates resulted in a remarkable reduction of protein translation activity. This decreased activity was obviously attributable to interaction with eIF3g, as the inhibition of translation was efficiently blocked by an additional amount of eIF3g, possibly due to competitive interaction. Although both AIF precursor and mature AIF interact with eIF3g in our binding experiments, AIF precursor containing MLS is known to be localized in the mitochondrial intermembrane space. Upon apoptosis induction, N-terminal MLS is removed by cleavage and mature AIF is released from mitochondria. In fact, mature AIF, but not precursor AIF, inhibits the protein translation significantly (data not shown), and it also activates caspase-7 better than precursor AIF as shown in Fig. 5D. Recently, another subunit of the eIF3 complex, eIF3f (p47), was observed to interact with cyclin-dependent kinase 11 (CDK11), and this interaction was found to be strengthened by the stimulation of apoptosis [28]. They showed that the caspase-processed C-terminal kinase domain of CDK11 resulted in the in vivo inhibition of the synthesis of a transfected luciferase reporter protein via interaction with eIF3f. Similarly, our data has revealed that AIF is definitely able to interact with eIF3g in the cytoplasm, especially in the perinuclear region (probably the ER) upon apoptotic stimulation, and that eIF3g can be one of the apoptotic target proteins that lose its functions during apoptotic process.

Whether protein synthesis is absolutely required for apoptosis to occur remains a matter of debate, and may vary based on stresses and cell types. In certain situations, de novo protein synthesis appears necessary for the induction of apoptosis. For example, p53-induced apoptosis has been demonstrated to require new protein synthesis for the full program to be completed [29]. It has also been reported that p53 results in the downregulation of overall translation at the level of polypeptide chain initiation, and p53 activation results in the caspase-independent downregulation of translation, along with the cleavage of eIF4GI and eIF4B, both of which are critical for protein synthesis [30,31]. Conversely, other reports have shown that apoptotic induction leads to the rapid caspase-dependent cleavage of initiation factors eIF4GI, eIF4GII, eIF4B, eIF2alpha, and the p35 subunit of eIF3 (eIF3j) [32–34]. A recent report has shown that protein synthesis is rapidly shut down in apoptosis, but not in necrosis, whereas translation initiation factors such as eIF4G and eIF2-α remain intact in necrosis, but not in apoptosis [35]. Interestingly, we found that the cleavage of eIF3g occurs in cells undergoing apoptosis after treatment with cisplatin and AIFmat. In several apoptotic cells, the polyclonal antibody against eIF3g has detected both the complete eIF3g (p44, 44 kDa) and its processed large fragments (30 kDa). Currently, we are examining eIF3g's caspase cleavage site, and the cellular localization of the cleaved eIF3g fragment. Importantly, cell death induced by AIF might be associated with a yet undefined function of eIF3g. It has recently been reported that the subcellular localization of eIF3g can change in apoptosis by anti-Fas stimulation and the knock-down of this protein may alter the apoptotic process [36]. In this study, we demonstrated that the cleavage of eIF3g occurs in cells undergoing apoptosis after the interaction of AIF with eIF3g followed by caspase activation. This observation strongly suggests that the translation inhibition of AIF is primarily mediated by interaction with eIF3g at an early time point, and that this inhibition of protein synthesis can be potentiated through the cleavage of eIF3g by caspase activation. Although the mechanism underlying caspase-7 activation

by AIFmat expression is not clear, our results shows that AIFmat-mediated inhibition of protein synthesis can be recovered by caspase-7 inhibitor in HEK293 and MCF-7 cells. In MCF-7 cells, it has been shown that AIFmat transfection also induces caspase-7 activation.

The idea that AIF can induce caspase-independent death is based on several key pieces of evidence. Some reports, however, suggest that crosstalk between AIF and caspases is possible. At least in some cases, AIF has been reported as an essential apoptotic factor released from mitochondria in the Cyt-*c*-dependent caspase activation cascade [37,38]. Recently, it has also been demonstrated that AIF can interact with HSP70, an inhibitor of Apaf-1-dependent caspase activation, indicating that another level of crosstalk may exist between AIF and caspases [17,39,40]. HSP70 is, therefore, the first cytoplasmic protein reported to interact with AIF, and its anti-apoptotic action may be attributed to AIF binding, which results in the cytosolic retention of AIF. HSP70, therefore, appears to be one of many candidates exhibiting anti-apoptotic functions. In another report, it has been suggested that AIF is capable of interacting and cooperating with cyclophilin A (CypA) to induce apoptosis-associated chromatinolysis [19]. It has been demonstrated that AIF translocates to the nucleus, where its interaction with CypA causes DNA degradation. Although this observation constitutes biochemical evidence that AIF induces chromatin condensation and DNA fragmentation, the AIF/CypA interaction cannot be the only factor determining the AIF-mediated regulation of cell death. In our study, we provide several lines of evidence supporting a role for AIF in protein translation and synthesis. Although a number of studies currently focus on the functional role of AIF in the nucleus, such as the induction of nuclease activity for DNA fragmentation, our observations reveal a new aspect of AIF-mediated apoptosis in the cytoplasm, prior to its translocation into the nucleus.

Taken together, this study demonstrates that AIF liberated from the mitochondria interacts with eIF3g, a subunit of the eIF3 complex, thereby inhibiting de novo protein synthesis, and finally activating caspase-7 for apoptosis amplification. Our data suggest that eIF3g is one of the target proteins for activated AIF, and our results also indicate that the modulation of translational activity constitutes a further mechanism by which the apoptotic effect of AIF may be mediated. Therefore, a detailed analysis of AIF's interaction with eIF might result in a clearer understanding of the functions of AIF, providing new insight into the mechanisms underlying apoptotic signaling and translational machinery collapse.

Acknowledgements: We are grateful to Dr. Tang K. Tang at Institute of Biomedical Sciences, Taiwan, for the generous gift of the eIF3g antibody. We also thank Dr. John W. Hershey (University of California, Davis, CA) for generously providing the anti-eIF3 antibody. This work was supported by grant from the Research Center for Women's Diseases of the KOSEF (R11-2005-017-03001) and by the Sookmyung Women's University Research Grants 2006.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2006.10.049.

References

[1] Steller, H. (1995) Mechanisms and genes of cellular suicide. Science 267, 1445–1449.
[2] Bergmann, A., Agapite, J. and Steller, H. (1998) Mechanisms and control of programmed cell death in invertebrates. Oncogene 17, 3215–3223.
[3] Cande, C., Cecconi, F., Dessen, P. and Kroemer, G. (2002) Apoptosis-inducing factor (AIF): key to the conserved caspase-independent pathways of cell death. J. Cell Sci. 115, 4727–4734.
[4] Susin, S.A., Zamzami, N., Castedo, M., Hirsch, T., Marchetti, P., Macho, A., Daugas, E., Geuskens, M. and Kroemer, G. (1996) Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. J. Exp. Med. 184, 1331–1341.
[5] Susin, S.A., Lorenzo, H.K., Zamzami, N., Marzo, I., Snow, B.E., Brothers, G.M., Mangion, J., Jacotot, E., Costantini, P., Loeffler, M., Larochette, N., Goodlett, D.R., Aebersold, R., Siderovski, D.P., Penninger, J.M. and Kroemer, G. (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 397, 441–446.
[6] Daugas, E., Susin, S.A., Zamzami, N., Ferri, K.F., Irinopoulou, T., Larochette, N., Prevost, M.C., Leber, B., Andrews, D., Penninger, J. and Kroemer, G. (2000) Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis. FASEB J. 14, 729–739.
[7] Susin, S.A., Daugas, E., Ravagnan, L., Samejima, K., Zamzami, N., Loeffler, M., Costantini, P., Ferri, K.F., Irinopoulou, T., Prevost, M.C., Brothers, G., Mak, T.W., Penninger, J., Earnshaw, W.C. and Kroemer, G. (2000) Two distinct pathways leading to nuclear apoptosis. J. Exp. Med 192, 571–580.
[8] Lorenzo, H.K., Susin, S.A., Penninger, J. and Kroemer, G. (1999) Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. Cell Death Differ. 6, 516–524.
[9] Miramar, M.D., Costantini, P., Ravagnan, L., Saraiva, L.M., Haouzi, D., Brothers, G., Penninger, J.M., Peleato, M.L., Kroemer, G. and Susin, S.A. (2001) NADH oxidase activity of mitochondrial apoptosis-inducing factor. J. Biol. Chem. 276, 16391–16398.
[10] Joza, N., Susin, S.A., Daugas, E., Stanford, W.L., Cho, S.K., Li, C.Y., Sasaki, T., Elia, A.J., Cheng, H.Y., Ravagnan, L., Ferri, K.F., Zamzami, N., Wakeham, A., Hakem, R., Yoshida, H., Kong, Y.Y., Mak, T.W., Zuniga-Pflucker, J.C., Kroemer, G. and Penninger, J.M. (2001) Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. Nature 410, 549–554.
[11] Polster, B.M., Basanez, G., Etxebarria, A., Hardwick, J.M. and Nicholls, D.G. (2005) Calpain I induces cleavage and release of apoptosis-inducing factor from isolated mitochondria. J. Biol. Chem. 280, 6447–6454.
[12] Loeffler, M., Daugas, E., Susin, S.A., Zamzami, N., Metivier, D., Nieminen, A.L., Brothers, G., Penninger, J.M. and Kroemer, G. (2001) Dominant cell death induction by extramitochondrially targeted apoptosis-inducing factor. FASEB J. 15, 758–767.
[13] Ye, H., Cande, C., Stephanou, N.C., Jiang, S., Gurbuxani, S., Larochette, N., Daugas, E., Garrido, C., Kroemer, G. and Wu, H. (2002) DNA binding is required for the apoptogenic action of apoptosis inducing factor. Nat. Struct. Biol. 9, 680–684.
[14] Vahsen, N., Cande, C., Dupaigne, P., Giordanetto, F., Kroemer, R.T., Herker, E., Scholz, S., Modjtahedi, N., Madeo, F., Le Cam, E. and Kroemer, G. (2006) Physical interaction of apoptosis-inducing factor with DNA and RNA. Oncogene 25, 1763–1774.
[15] Yu, S.W., Wang, H., Poitras, M.F., Coombs, C., Bowers, W.J., Federoff, H.J., Poirier, G.G., Dawson, T.M. and Dawson, V.L. (2002) Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 297, 259–263.
[16] Matsumori, Y., Hong, S.M., Aoyama, K., Fan, Y., Kayama, T., Sheldon, R.A., Vexler, Z.S., Ferriero, D.M., Weinstein, P.R. and Liu, J. (2005) Hsp70 overexpression sequesters AIF and reduces neonatal hypoxic/ischemic brain injury. J. Cereb. Blood Flow Metab. 25, 899–910.
[17] Ravagnan, L., Gurbuxani, S., Susin, S.A., Maisse, C., Daugas, E., Zamzami, N., Mak, T., Jaattela, M., Penninger, J.M., Garrido, C. and Kroemer, G. (2001) Heat-shock protein 70 antagonizes apoptosis-inducing factor. Nat. Cell Biol. 3, 839–843.

[18] Schmitt, E., Parcellier, A., Gurbuxani, S., Cande, C., Hammann, A., Morales, M.C., Hunt, C.R., Dix, D.J., Kroemer, R.T., Giordanetto, F., Jaattela, M., Penninger, J.M., Pance, A., Kroemer, G. and Garrido, C. (2003) Chemosensitization by a non-apoptogenic heat shock protein 70-binding apoptosis-inducing factor mutant. Cancer Res. 63, 8233–8240.
[19] Cande, C., Vahsen, N., Kouranti, I., Schmitt, E., Daugas, E., Spahr, C., Luban, J., Kroemer, R.T., Giordanetto, F., Garrido, C., Penninger, J.M. and Kroemer, G. (2004) AIF and cyclophilin A cooperate in apoptosis-associated chromatinolysis. Oncogene 23, 1514–1521.
[20] Browning, K.S., Gallie, D.R., Hershey, J.W., Hinnebusch, A.G., Maitra, U., Merrick, W.C. and Norbury, C. (2001) Unified nomenclature for the subunits of eukaryotic initiation factor 3. Trends Biochem. Sci. 26, 284.
[21] Block, K.L., Vornlocher, H.P. and Hershey, J.W. (1998) Characterization of cDNAs encoding the p44 and p35 subunits of human translation initiation factor eIF3. J. Biol. Chem. 273, 31901–31908.
[22] Asano, K., Vornlocher, H.P., Richter-Cook, N.J., Merrick, W.C., Hinnebusch, A.G. and Hershey, J.W. (1997) Structure of cDNAs encoding human eukaryotic initiation factor 3 subunits. Possible roles in RNA binding and macromolecular assembly. J. Biol. Chem. 272, 27042–27052.
[23] Mengod, G. and Trachsel, H. (1985) Eukaryotic protein synthesis initiation factor eIF-3: determination of concentration and association with ribosomes in rabbit reticulocyte and HeLa cell lysates. Biochim. Biophys. Acta 825, 169–174.
[24] Milburn, S.C., Duncan, R.F. and Hershey, J.W. (1990) Immunoblot analysis of the structure of protein synthesis initiation factor eIF3 from HeLa cells. Arch. Biochem. Biophys. 276, 6–11.
[25] Kolupaeva, V.G., Unbehaun, A., Lomakin, I.B., Hellen, C.U. and Pestova, T.V. (2005) Binding of eukaryotic initiation factor 3 to ribosomal 40S subunits and its role in ribosomal dissociation and anti-association. RNA 11, 470–486.
[26] Park, H.S., Browning, K.S., Hohn, T. and Ryabova, L.A. (2004) Eucaryotic initiation factor 4B controls eIF3-mediated ribosomal entry of viral reinitiation factor. EMBO J. 23, 1381–1391.
[27] Hou, C.L., Tang, C., Roffler, S.R. and Tang, T.K. (2000) Protein 4.1R binding to eIF3-p44 suggests an interaction between the cytoskeletal network and the translation apparatus. Blood 96, 747–753.
[28] Shi, J., Feng, Y., Goulet, A.C., Vaillancourt, R.R., Sachs, N.A., Hershey, J.W. and Nelson, M.A. (2003) The p34cdc2-related cyclin-dependent kinase 11 interacts with the p47 subunit of eukaryotic initiation factor 3 during apoptosis. J. Biol. Chem. 278, 5062–5071.
[29] Polyak, K., Xia, Y., Zweier, J.L., Kinzler, K.W. and Vogelstein, B. (1997) A model for p53-induced apoptosis. Nature 389, 300–305.
[30] Constantinou, C., Bushell, M., Jeffrey, I.W., Tilleray, V., West, M., Frost, V., Hensold, J. and Clemens, M.J. (2003) p53-induced inhibition of protein synthesis is independent of apoptosis. Eur. J. Biochem. 270, 3122–3132.
[31] Horton, L.E., Bushell, M., Barth-Baus, D., Tilleray, V.J., Clemens, M.J. and Hensold, J.O. (2002) p53 activation results in rapid dephosphorylation of the eIF4E-binding protein 4E-BP1, inhibition of ribosomal protein S6 kinase and inhibition of translation initiation. Oncogene 21, 5325–5334.
[32] Marissen, W.E. and Lloyd, R.E. (1998) Eukaryotic translation initiation factor 4G is targeted for proteolytic cleavage by caspase 3 during inhibition of translation in apoptotic cells. Mol. Cell Biol. 18, 7565–7574.
[33] Bushell, M., Poncet, D., Marissen, W.E., Flotow, H., Lloyd, R.E., Clemens, M.J. and Morley, S.J. (2000) Cleavage of polypeptide chain initiation factor eIF4GI during apoptosis in lymphoma cells: characterisation of an internal fragment generated by caspase-3-mediated cleavage. Cell Death Differ. 7, 628–636.
[34] Clemens, M.J., Bushell, M., Jeffrey, I.W., Pain, V.M. and Morley, S.J. (2000) Translation initiation factor modifications and the regulation of protein synthesis in apoptotic cells. Cell Death Differ. 7, 603–615.
[35] Saelens, X., Festjens, N., Parthoens, E., Vanoverberghe, I., Kalai, M., van Kuppeveld, F. and Vandenabeele, P. (2005) Protein synthesis persists during necrotic cell death. J. Cell Biol. 168, 545–551.
[36] Machuy, N., Thiede, B., Rajalingam, K., Dimmler, C., Thieck, O., Meyer, T.F. and Rudel, T. (2005) A global approach combining proteome analysis and phenotypic screening with RNA interference yields novel apoptosis regulators. Mol. Cell. Proteomics 4, 44–55.
[37] Arnoult, D., Gaume, B., Karbowski, M., Sharpe, J.C., Cecconi, F. and Youle, R.J. (2003) Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization. EMBO J. 22, 4385–4399.
[38] Arnoult, D., Parone, P., Martinou, J.C., Antonsson, B., Estaquier, J. and Ameisen, J.C. (2002) Mitochondrial release of apoptosis-inducing factor occurs downstream of cytochrome c release in response to several proapoptotic stimuli. J. Cell Biol. 159, 923–929.
[39] Gurbuxani, S., Schmitt, E., Cande, C., Parcellier, A., Hammann, A., Daugas, E., Kouranti, I., Spahr, C., Pance, A., Kroemer, G. and Garrido, C. (2003) Heat shock protein 70 binding inhibits the nuclear import of apoptosis-inducing factor. Oncogene 22, 6669–6678.
[40] Garrido, C., Schmitt, E., Cande, C., Vahsen, N., Parcellier, A. and Kroemer, G. (2003) HSP27 and HSP70: potentially oncogenic apoptosis inhibitors. Cell Cycle 2, 579–584.